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Trace elements bioaccumulation in liver and fur of *Myotis myotis* from two caves of the eastern side of Sicily (Italy): A comparison between a control and a polluted area☆

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ABSTRACT

Environmental pollution is a topic of great interest because it directly affects the quality of ecosystems and of all living organisms at different trophic and systematic levels. Together with the global climate change, the long-term surviving of many species of plants and animals is threaten, distributional patterns at global and regional levels are altered and it results in local assemblages of species that are quite different from those that currently constitute coevolved communities. .For this study, the species *Myotis myotis* was used as bioindicator and it was sampled from two caves in the south-east of Sicily, Pipistrelli chosen as control area and Palombara chosen as polluted area, to measure the concentrations of trace elements in fur and liver tissues. Results showed higher content of essential elements in fur in bats sampled from Pipistrelli. Conversely, higher concentrations of toxic metals in liver such as As, Cd, Pb and Hg were measured in bat samples in Palombara cave, where specimens have a hunting area extended within the boundaries of the petrochemical plant. Nevertheless, we cannot consider Palombara population as polluted by metal contamination since their tissue concentrations are overall lower than toxic thresholds values suggested for small mammals. Likewise, we cannot exclude other kind of pollutants as potential stressors of the examined population, contributing with the decreasing of bat colonies in Sicily.

1. Introduction

Environmental pollution is a topic of great interest because it directly affects the quality of ecosystems and of all living organisms at different trophic and systematic levels (Ferrante et al., 2017b). Pollutants can be directly uptaken by organisms through ingestion of contaminated prey items, dermal exposure or inhalation. One of the most effective approaches for evaluating metals in the environment, and thus of the risk associated for organisms living in a metal contaminated environment, is the biological monitoring (Ferrante et al., 2015; Mazzei et al., 2014). From this perspective, it is crucial to assess the concentrations of contaminants bioaccumulated in organisms to understand their potential adverse effects, especially of long-term persistent chemicals such as metals. The most toxic metals are As, Cd, Pb and Hg, which represent the ultimate form of persistent pollutants. Unlike other pollutants, metals are not degraded in the environment and can accumulate through the food chain posing potential risks to the human health and the health of ecosystems (Mazzei et al., 2014). Nevertheless, some metals are essential for organisms and are naturally available in food and water, but in the case of high levels or lack of these essential elements (e.g., manganese, nickel, cobalt, copper, iron, and zinc), adverse health effects may occur (Ferrante et al., 2018; Mansour et al., 2016).

Insectivorous bats are considered reliable bioindicator organisms as: 1) they are vulnerable to a wide range of environmental stressors; 2) they are long-lived mammals and occupy higher

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positions in the trophic chain; 3) they occur on a wide geographic range; 4) their population dynamics can present rapid declines due to their slow reproductive rates; 5) they provide key ecosystem services; 6) they are more likely to show the effects of pollutants than fructivorous bats (Alleva et al., 2006; Jones et al., 2009; Zukal et al., 2015).

Furthermore, due to their relatively long life, up to 37 years recorded for *Myotis brandtii* (Gaisler et al., 2003), compared to their small body size, and their high daily food intake (e.g. up to 0.5 g/bw/day on a wet basis measured experimentally for *Myotis lucifugus*) (Anthony and Kunz, 1977), bats can be particularly prone to chemical exposure, especially to contaminants such as metals accumulated through the food chain (Hernout et al., 2016a). The chemical contamination is one of many stressors, which is implicated in the demographic decline of many bat populations across Europe and North America, but the risks to bats remain poorly understood (Hernout et al., 2016a). To biomonitor environmental contamination in wildlife species, non-invasive proxy have been proposed and investigated such as feathers and fur. Concentrations of pollutants in fur reflect the exposure during the period in which the hair is growing, which occurs once annually between summer and fall (Fraser et al., 2013). Bat fur can be a reliable non-invasive tool to investigate metal contamination, however, the method has been evaluated on a limited number of metals (Hernout et al., 2016b). In addition, and with the focus mainly on long-term exposure, adipose tissue and other tissues like bone, liver, brain or kidney, were used as long-term biomonitors of certain elements/metals (Domingo et al., 2017). *Myotis myotis*, the greater mouse-eared bat, is an European species included in the Red List as Least Concern 3.1 (IUCN, 2017) and Vulnerable A2c in the Red List of Italian Vertebrate (IUCN-Italy, 2013). *M. myotis* is widely distributed in Europe and the Mediterranean area and is an insectivorous species with an average life expectancy ranging from 2.4 to 2.7 to 4–5 years, with a maximum longevity of 22 years (Dietz et al., 2009). In Italy, the *M. myotis* population is stable and occurs on the entire territory (Lanza and Agnelli, 1999). However, in Sicily, the insular population of *M. myotis* decreased and several colonies disappeared compared to the past decades (Agnelli et al., 2004). Whereas the causes of the population decline of *M. myotis* in Sicily have not been identified, further research is needed to investigate the potential causes of declines of bat population. Several studies have shown metal exposure and accumulation in bat tissues (Hernout et al., 2016a; Lisón et al., 2017) which could be contributing to the decline of bat populations in Sicily, and should therefore be further evaluated.

In this study, we choose to focus on two sites in Sicily. One used as a control site and one as polluted site, based on its proximity (1 km) to a petrochemical plant (Fig. 1). In this polluted area, the industrial activities started in the late 1950s and 1960s and contain several oil refineries, chemical plants, mineral deposits, a military base and many other industrial installations. During the last few decades, the industrial activities have caused progressive contamination of the different environmental matrices through the presence of compounds that are mainly toxic, persistent and can bioaccumulate. The marine environment across the petrochemical plant has been more studied than the terrestrial environment and the overall results demonstrated a severe As, Cd, Cr, Hg, Pb, polychlorobiphenyls (PCBs) and polycyclic aromatic hydrocarbons (PAHs) contamination. The concentrations determined in sediments and seafood were exceeding the standard limit reported by national and international sediment quality guidelines (SQGs) (Ministerial Decree No. 260/2010; (De Domenico et al., 2013) and the standard limit for human consumption (D.lgs n.1881/2006), respectively. DNA damage, reduced genetic variability and oxidative stress were observed in marine organisms (Ausili et al., 2008; De Domenico et al., 2011; Di Leonardo et al., 2009; Ferrante et al., 2017b; Longo et al., 2013; Romano et al., 2016; Tomasello et al., 2012). The Italian National Institute of Health evaluated the toxicological properties of the dangerous substances in the petrochemical area and emphasized the correspondence between the types of contaminants and soil, groundwater and sediments. The recognition of an area as being at high risk entails priority allocation of resources, for cleaning up contamination and for rehabilitation in general.

The aim of this study was: 1) to assess the bioaccumulation of Arsenic (As), Cadmium (Cd), Cobalt (Co), Chromium (Cr), Copper (Cu), Mercury (Hg), Manganese (Mn), Nickel (Ni), Lead (Pb), Selenium (Se), Antimony (Sb), and Vanadium (V) in fur and liver of individuals of *M. myotis* sampled from two different caves used as nursery roosts in Sicily; 2) to compare the amount of metals accumulated in bat tissues between the two sites; 3) to compare the levels of metals with toxic thresholds for toxic metals and upper range values for essential metals in small mammals; and 4) to establish positive and negative correlations between metal concentrations determined in fur and liver. The present study is the first monitoring survey of trace elements in bats carried out in Italy.

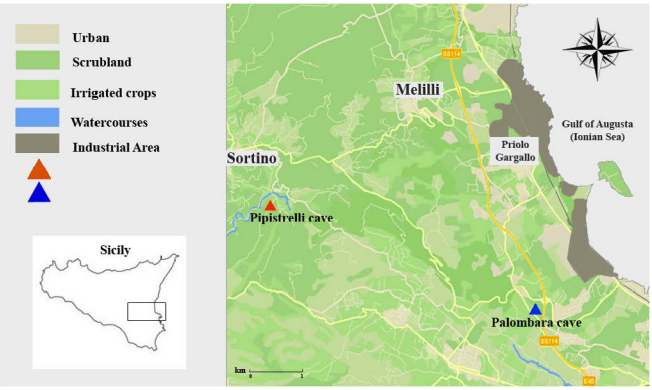

Fig. 1. Map showing the study areas (southern Italy – Sicily). In particular, the triangles indicate the geographic position of the caves and the color gradations indicate the different exploitation of the territory.

2. Material and methods

2.1. Sampling area

The exposure of bats to metals was compared between two caves. Pipistrelli was chosen as control area and Palombara as polluted area since it is located at 1 km to a petrochemical plant of southern Italy (Fig. 1).

The control site is located in the Pantalica area, awarded in 2005 as UNESCO world heritage site for its history, archeology, speleology and landscape. The area is characterized by a nature plateau, deeply engraved by the quarries of the Anapo valley and the Calcinara river. Rocks are predominantly calcareous but we also found volcanoclastites, minor lava flows and diatremes (maars) associated with biocalcarenite deposits (Grasso and Lentini, 1982; Savelli, 2001). The site is a variable of natural and semi-natural habitats along with arable land, which are essential habitats for the invertebrate and vertebrate communities. Pipistrelli cave is an important bat cave in Sicily since it hosts the largest colonies of bats and it represents the biggest nursery roost of Sicily (Spena et al., 2013). The following *taxa* were observed: *Rhinolophus ferrumequinum*, *R. euryale*, *R. hipposideros*, *Myotis myotis* vel *blythii*, *M. capaccinii* and *Miniopterus schreibersii* (Grasso et al., 2013; Spena et al., 2013). The maximum number of occurrences (taking into account all the

species together) was recorded in summer (over 9000 in July 2013). The presence of numerous juveniles in summer suggests that colonies from the Pipistrelli cave are primarily reproductive colonies (Grasso et al., 2013; Spena et al., 2013).

Palombara cave is of karstic origin, located in the natural reserve of Palombara, in Climiti mounts, and is located near the biggest petrochemical plant of Italy, known as "Augusta-Priolo-Melilli". In this area, there is the formation of the Climiti Mountains of the Oligo-Miocene age, characterized by calcirudite and whitish calcarenite. The area looks like a vast plateau with extensive rocky outcrops. There are also doliniform soil depressions, which in the winter season allow the accumulation of rainwater, transforming them into humid environments. The cave hosts a colony of bats belonging to the species: *Myotis myotis, Miniopterus schreibersii, Rhinolophus euryale, Rhinolophus ferrumequinum*. The maximum number of Chiroptera recorded in the summer was about 1000 specimens (Spena et al., 2013). The environment around the cave has undergone strong anthropogenic modifications and is degraded due to the presence of numerous illegal landfills.

The habitat around Pipistrelli cave is suitable for many bat species as listed above. In contrast, the progressive isolation and degradation of the environment surrounding Palombara cave is probably one of the main causes for the decline of the colony. Although the current metal concentrations in soil or sediments were not determined for both study sites, the characterization of Palombara as polluted and Pipistrelli as control site is suitable for the purpose of this study. The ideal foraging area for bats is near to the roost, but sometimes the animals have to fly for many kilometres. The maximum recorded distance changes according to the morphological characteristics of the wings and to the different sonar system adopted by the species. The hunting grounds of *Myotis myotis* lie usually in 5–15 km periphery around the roost (Dietz et al., 2009). The hunting grounds for *M. myotis* are characterized by forested areas (mainly deciduous and mixed woodlands) with free access to the ground (meadows, pastures and fields), as this bat species forages on ground-dwelling arthropods (mainly beetles, chilopods, spiders, grasshoppers). These habitats are present nearby both caves, so we cannot exclude some occasional mingling between the two colonies living in the Pipistrelli cave and in Palombara cave, separated by 15 km only. However, considering the high level of fidelity exhibited by the bats roosting in permanent refuge like the caves (Kunz and Fenton, 2003) and particularly the strong fidelity of the young bats to the colony and of the adults to the nursery during the breeding season, these events of roost switching are negligible.

2.2. Sampling and sample treatment

In the spring of 2013 and 2014, 101 dead bats were collected in Pipistrelli and Palombara caves in Sicily, Italy. 51 adults (34 males and 17 females) of *M. myotis* from Pipistrelli cave and 50 adults (24 males and 26 females) were collected from Palombara. Hundreds of dead specimens have been found in these caves (Salvaggio et al., 2013), showing no apparent trauma, regardless of age and gender and the state of conservation of the carcasses. The specimens chosen for sampling were in a relative good state of conservation. After sampling, the specimens were transported in ice to the laboratory where fixed aliquots were weighted to ensure homogeneity of samples. In particular, 0.25 ± 0.15 g of dorsal fur and 0.45 ± 0.17 liver (entire liver) were excised using a stainless steel razor, thoroughly washed with Bi-distilled water. Fur sample was repeatedly washed with ultra-pure water, to avoid external contamination of metals, until the washing water was clean (Hernout et al., 2016b). In a small number of fur samples, a sonication (Digit, ISCO) with ultrapure water for 3 min was needed until a clear cleaning solution was obtained. Then, fur was dried until constant weight at 60 °C overnight. Samples were then preserved at −80 °C until processing.

2.3. Heavy metal analysis

Metal concentrations were determined for each samples, and therefore for each bat. The samples were weighed with an analytical grade balance and then digested with a microwave (Milestone, Ethos TC) using a mixture of strong acids. A digestion solution was prepared with 6 mL of 65% nitric acid (HNO_3-Carlo Erba, Italy) and 2 mL of 30% peroxide hydrogen (H_2O_2-Carlo Erba, Italy). Samples were digested for 50 min at 120 °C. After mineralization, the vessels were opened if a temperature <22 °C was reached to avoid loss of volatile elements, then the content was decanted and ultra-pure water (Merck, USA) was added to the samples up to 50 mL. In aliquots of 10 mL of digested samples were added 50 $\mu g\ L^{-1}$ of internal standard (Yttrium, Y, and Rhenium, Re, Merck, USA) for quantification of metals with an Inductively Coupled Plasma - Mass Spectrometer (ICP-MS) Elan-DRC-e (Perkin Elmer, USA). The operating parameters included an ICP radio frequencies power of 1.30 kW, a plasma gas (argon) flow of 15 L/min, an auxiliary gas flow for the Standard mode of 1.20 L/min (argon), an auxiliary gas flow for the Dynamic Reaction Cell-DRC mode (methane) of 0.88 L/min, a Nebulizer Gas Flow of 0.96 L/min. Isotopes (m/z) monitored in Standard Mode are V^{50}, Cr^{52}, Mn^{55}, Co^{59}, Ni^{60}, Cu^{62}, Cd^{114}, Sb^{120}, Hg^{202}, Pb^{208}; isotopes monitored in DRC mode are As^{75}, Se^{78}; isotopes of internal standards are Y^{89}, Re^{187}.

Concentrations were determined using standard solutions prepared in the same acid matrix. Standards for the instrument calibration were prepared based on mono element certified reference solution ICP Standard (Merck, USA). The quantification method compares the analyte signals and the internal standard signals. The calibration curve was used to quantify concentration values based on the analyte signals.

Analytical blanks were processed in the same way as samples. For the quality control, human hair samples or heavier liver samples were prepared in duplicate with homogeneous weights for each batch of mineralization. For each duplicate, one was spiked with a multi-elements solution of 25 mg/L with 5 mg/kg and we obtained a mean recovery of 89% As, 91% Cd, 110% Co, 92% Cr, 113% Cu, 82% Hg, 95% Mn, 109% Ni, 86%, Pb, 81% Se, 97% Sb and 97% V. The limits of detection (LOD) were calculated based on the 40 CFR 136, EPA procedure (US-EPA, 2016) for digested analytical blanks using the following equation:

$$LOD = t\ (df = n\text{-}1,\ p = 0.99\%) \times SD$$

where t is the one tail student's t distribution, df are the degree of freedom, n the number of blank replicates, p is the probability and SD the standard deviations.

The limits of detection (LOQ) were calculated based on the IUPAC procedure (IUPAC, 2014) for digested analytical blanks using the following equation:

$$LOQ = 10SD + Xm$$

where Xm is the mean of the blank measures.

LOD obtained were (in mg/kg): 0.01 As, 0.01 Cd, 0.005 Co, 0.07 Cr, 0.1 Cu, 0.013 Hg, 0.05 Mn, 0.02 Ni, 0.02 Pb, 0.02 Se, 0.014 Sb and 0.012 V.

LOQ obtained were (in mg/kg): 0.09 As, 0.08 Cd, 0.05 Co, 0.61 Cr, 0.21 Cu, 0.15 Hg, 0.52 Mn, 0.18 Ni, 0.22 Pb, 0.24 Se, 0.16 Sb and 0.14 V.

Table 1
Descriptive statistic and Mann-Whitney *U* test of trace elements analysis in fur (mg/Kg dry weight) and liver (mg/kg wet weight) of *M. myotis* sampled from Palombara and Pipistrelli caves.

Fur-Palombara		As	Cd	Co	Cr	Cu	Hg	Mn	Ni	Pb	Se	Sb	V
N.		50	50	50	50	50	50	50	50	50	50	50	50
Mean		0.643	0.015	0.032	1.054	5.468	2.372	2.914	0.426	0.763	0.807	0.057	0.292
Median		0.434	0.015	0.023	0.868	5.206	1.821	2.193	0.249	0.240	0.748	0.044	0.298
SD		0.670	0.012	0.020	0.910	1.749	1.695	2.791	0.568	1.074	0.347	0.044	0.179
Minimum		<0.01	<0.01	0.012	0.299	1.449	0.632	0.507	<0.02	0.050	0.306	<0.01	<0.01
Maximum		4.099	0.075	0.094	5.080	9.470	9.409	18.647	3.434	6.086	1.698	0.178	0.729
Percentiles	25	0.305	<0.01	0.018	0.627	4.363	1.272	1.213	0.123	0.114	0.524	0.022	0,154
	50	0.434	<0.01	0.023	0.868	5.206	1.821	2.193	0.249	0.240	0.748	0.044	0.298
	75	0.708	0.015	0.043	1.071	6.745	2.954	4.106	0.553	1.039	1.101	0.082	0,384
Fur-Pipistrelli		As	Cd	Co	Cr	Cu	Hg	Mn	Ni	Pb	Se	Sb	V
N.		51	51	51	51	51	51	51	51	51	51	51	51
Mean		0.680	0.016	0.086	1.142	7.760	2.741	3.386	0.564	0.432	1.081	0.068	0.453
Median		0.554	0.014	0.061	0.987	7.349	2.458	2.917	0.433	0.287	1.061	0.054	0.427
SD		0.453	0.007	0.122	0.565	1.865	2.376	2.320	0.484	0.353	0.265	0.045	0.216
Minimum		<0.01	<0.01	0.011	0.466	3.797	0.170	0.518	0.036	0.037	0.529	<0.01	0.113
Maximum		2.182	0.037	0.877	3.204	12.508	10.285	11.101	3.090	1.707	1.706	0.215	1.163
Percentiles	25	0.359	<0.01	0.032	0.787	6.357	1.384	1.647	0.308	0.182	0.918	0.036	0.266
	50	0.554	0.014	0.061	0.987	7.349	2.753	2.917	0.433	0.287	1.061	0.054	0.427
	75	0.863	0.020	0.097	1.373	8.976	3.145	4.616	0.635	0.631	1.265	0.093	0.606
Mann-Whitney *U* test		As	Cd	Co	Cr	Cu	Hg	Mn	Ni	Pb	Se	Sb	V
Fur vs Fur p-value		0.129	0.110	0.000	0.037	0.000	0.112	0.137	0.002	0.884	0.000	0.131	0.000
Liver-Palombara		As	Cd	Co	Cr	Cu	Hg	Mn	Ni	Pb	Se	Sb	V
N.		47	47	47	47	47	44	47	47	47	47	47	47
Mean		0.049	0.335	0.104	0.983	10.302	1.792	10.235	0.079	0.097	0.820	<0.01	0.050
Median		0.032	0.284	0.091	0.880	7.499	0.745	8.959	0.032	0.063	0.677	<0.01	0.040
SD		0.077	0.308	0.059	0.788	7.578	2.215	5.035	0.106	0.126	0.438	0.001	0.026
Minimum		<0.01	0.013	0.034	<0.07	4.828	0.184	2.357	<0.02	<0.02	0.268	<0.01	0.014
Maximum		0.530	1.333	0.231	2.710	48.741	10.175	22.038	0.449	0.850	1.870	0.018	0.110
Percentiles	25	0.022	0.079	0.064	0.256	6.645	0.385	7.572	0.020	0.049	0.527	<0.01	0.031
	50	0.032	0.284	0.091	0.880	7.499	0.745	8.959	0.032	0.063	0.677	<0.01	0.040
	75	0.051	0.464	0.148	0.993	11.458	2.683	13.410	0.088	0.112	0.925	<0.01	0.063
Liver-Pipistrelli		As	Cd	Co	Cr	Cu	Hg	Mn	Ni	Pb	Se	Sb	V
N.		50	50	50	50	50	50	50	50	50	50	50	50
Mean		0.028	0.243	0.093	0.883	9.255	1.495	10.015	0.039	0.064	0.987	<0.01	0.045
Median		0.028	0.088	0.082	0.879	8.192	0.515	8.321	0.021	0.037	0.887	<0.01	0.029
SD		0.023	0.349	0.049	0.606	3.857	2.601	5.130	0.049	0.066	0.475	<0.01	0.037
Minimum		<0.01	<0.01	0.036	0.179	4.825	0.037	3.162	<0.02	<0.02	0.342	<0.01	<0.01
Maximum		0.183	1.495	0.241	2.386	22.764	11.811	24.447	0.259	0.308	2.653	0.015	0.134
Percentiles	25	0.014	0.043	0.051	0.254	7.037	0.312	5.950	<0.02	0.025	0.607	<0.01	0.015
	50	0.023	0.088	0.082	0.879	8.192	0.515	8.321	0.021	0.037	0.887	<0.01	0.029
	75	0.045	0.327	0.121	1.178	10.037	1.184	12.376	0.042	0.081	1.267	<0.01	0.081
Mann-Whitney *U* test		As	Cd	Co	Cr	Cu	Hg	Mn	Ni	Pb	Se	Sb	V
Liver vs Liver p-value		0.002	0.016	0.382	0.885	0.525	0.037	0.603	0.025	0.045	0.041	0.094	0.035

2.4. Statistical analysis

The software IBM SPSS 20.0 was used for the statistical analysis. Results below the limit of detection were elaborated as LOD/2. Overall, less than 5% of As, Cr, Pb and V concentrations were found below the LOD values. 50% of Cd concentrations measured in fur samples were found below LOD value, Ni and Sb concentrations measured in liver samples were found below the LOD values in 28% and 94% of the liver samples, respectively.

The normal distribution was verified using the Kolmogorov–Smirnov test. Since V, Co, Ni, Cu, Se and Hg concentrations did not have a normal distribution in fur, and V, As, Se and Cd concentrations in liver, the Mann-Whitney non-parametric test was used to compare median concentrations of trace elements between sample sites (liver and fur of the control population versus liver and fur of the exposed population) and sex (male versus female of the same population). To compare liver concentration with literature data, raw liver data were converted in dry weight applying a conversion factor of 4 (Ma, 1994). Spearman correlation test was applied to evaluate the strength of the relationships between metal concentrations measured in fur and liver for each cave.

3. Results

3.1. Trace element concentrations in bats: comparison between the sites

101 bats collected in Sicily were analysed for metal analysis. The monitoring study presents metal concentrations in bats from two different caves, a control site versus a polluted site (Table 1). Overall, metal concentrations in fur samples showed a similar trend in both caves: with the highest concentrations (exceeding 0.8 mg/

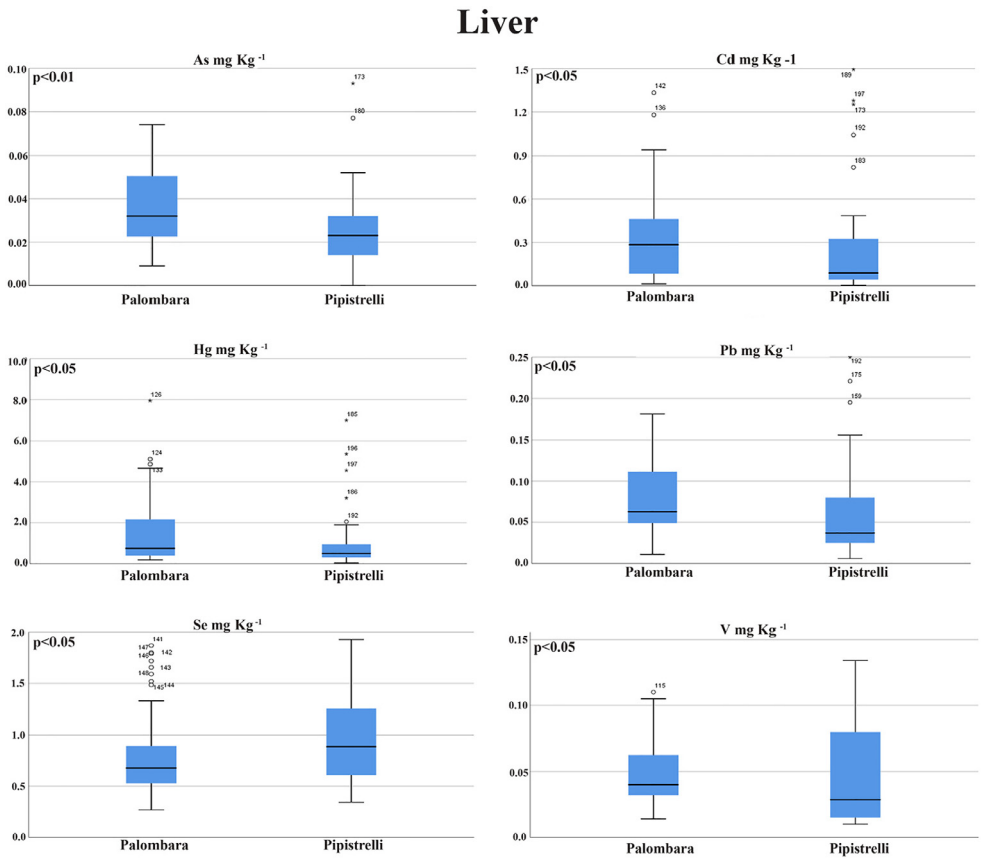

Fig. 2. Box Plot of metal concentrations (mg/kg wet weight) bioaccumulated in liver of *M. myotis* with significant differences between Palombara and Pipistrelli caves. P-values "p" given by the Mann-Whitney *U* test are presented in the figure.

kg dry weight) as follows Cu > Mn > Hg > Cr > Se (Table 1). Metal concentrations in liver tissues were overall the highest (exceeding 0.8 mg/kg wet weight) for Cu, Mn, Hg, Cr and Se (Table 1).

Hepatic concentrations of toxic elements and non-essential elements (As, Cd, Hg, and Pb) were higher in samples of the polluted compared to the control site ($p < 0.001$ for As and $p < 0.05$ for the others metals) (Table 1, Fig. 2). Similar results were observed for V concentrations ($p < 0.05$). Se was found significantly higher in liver of bats from the Pipistrelli cave than in bats from the Palombara cave ($p < 0.05$) (Table 1, Fig. 2). Although the mean hepatic concentrations of the other metals were higher for the bats sampled in Palombara than for the bats sampled in Pipistrelli, the differences were not significant (Table 1).

For trace elements bioaccumulated in fur, concentrations of Cr ($p < 0.05$), Ni ($p < 0.01$), Co, Cu, Se and V ($p < 0.001$) in specimens sampled in the Pipistrelli cave were significantly higher than the concentrations determined in bats sampled in the Palombara cave (Table 1; Fig. 3). For all other metals analysed in fur, essential (Mn) and non-essential (As, Cd, Sb, Hg and Pb) concentrations were comparable between the caves. Furthermore, we did not find any difference in metal bioaccumulation between sex by comparing trace element concentrations for each tissue and for the same cave.

The strength of the associations between concentrations of trace metals in fur versus liver were assessed. Toxic metals (As, Hg and Pb) concentrations in liver and in fur were positively correlated in bats sampled from Pipistrelli, with the strongest correlation found for Hg, followed by As and Pb (Hg: $rho = 0.627$, $p < 0.001$; As: $rho = 0.458$, $p < 0.01$; Pb: $rho = 0.301$, $p < 0.05$) (Table 2, Fig. 4). Other positive correlations between tissues were found for V in both caves (Pipistrelli: $rho = 0.492$, $p < 0.00$; Palombara: $rho = 0.387$, $p < 0.01$) and Se in Pipistrelli ($rho = 0.434$, $p < 0.05$) (Table 2, Fig. 4).

Spearman's coefficient revealed a significant negative correlation for Cr (Pipistrelli: $rho = -0.399$, $p < 0.05$), and Cd (Palombara: $rho = -0.388$, $p < 0.01$) concentrations (Table 2, Fig. 4).

4. Discussion

4.1. Trace element concentrations in bat: comparison between the sites

Concentrations of trace elements were determined in *M. myotis* tissues from a polluted site and a control site. Results revealed a higher content of toxic metals in liver of bats from Palombara versus a higher content of essential elements in fur of bats from Pipistrelli.

Liver is key organ in detoxification processes and it is used as a biomarker of potential environmental contamination since exogenous compounds can accumulate in this organ (Wijnhoven et al., 2007). For example, ingested Cd is absorbed by intestinal cells and transported by blood flow to the liver (Reis et al., 2010). When animals are acutely Pb poisoned, this mineral is deposited specially in liver (Miranda et al., 2006; Rumbeiha et al., 2001; Swarup et al., 2007; Waldner et al., 2002), while, in chronic poisoning, lead deposition occurs in bones (Reis et al., 2010). Metal concentrations in hepatic tissues of bats from Palombara cave showed a higher accumulation of As, Cd, Hg, Pb, and V. The toxicity of V depends on its chemical forms and oxidation states (Arena et al., 2015; Mukherjee et al., 2004). Thus, the data presented in this study suggest a higher chronic and long-term exposure of toxic metals in

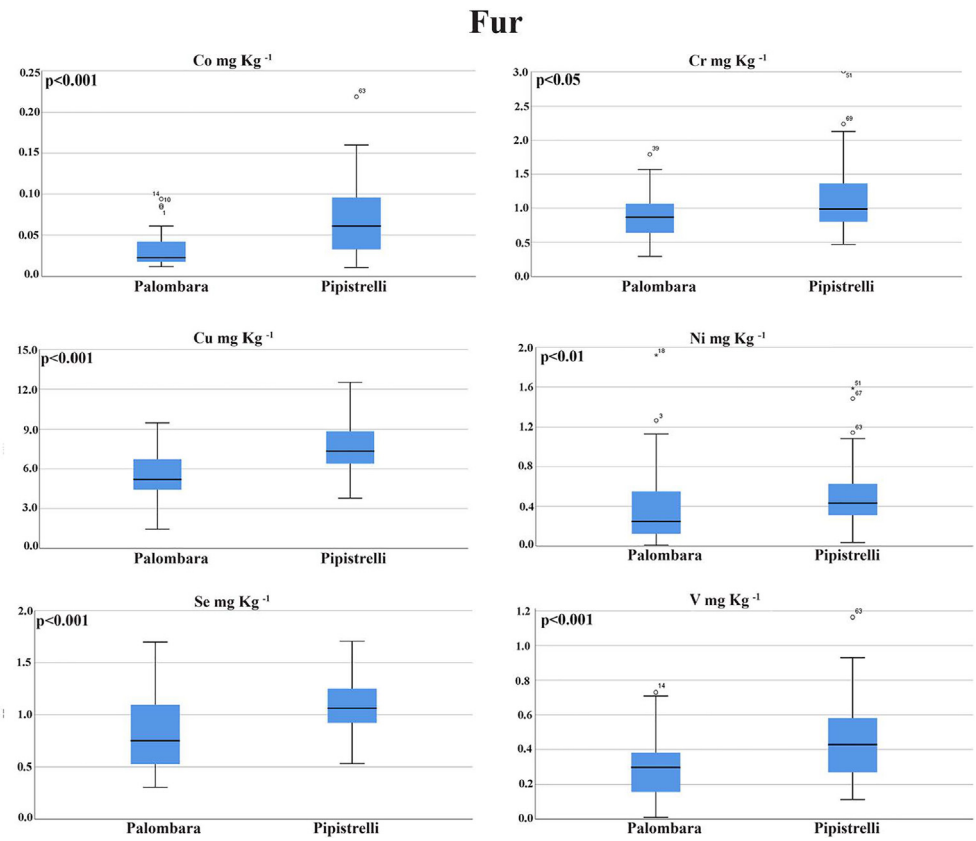

Fig. 3. Box Plot of metal concentrations (mg/kg dry weight) bioaccumulated in fur of *M. myotis* with significant differences between Palombara and Pipistrelli caves. P-values "p" given by the Mann-Whitney *U* test are presented in the figure. Trace element correlations between fur versus liver.

bats from Palombara than in the control site.

Several toxic effects could be caused by the aforementioned metals, if their internal thresholds are exceeded. Arsenic has been confirmed as a human carcinogen and recent studies have also suggested a relationship with diabetes, neurological effects, cardiac disorders, and reproductive organs (Hong et al., 2014). Cd increases reactive oxygen species (ROS) and clinical symptoms of Cd poisoning in mammals are reduction in growth and weight gain food intake, anemia renal changes, less development of testes or testicular degeneration, abortion and tumors (Reis et al., 2010). The exposure to Pb induces toxicity occurring in kidney and endocrine system as well as reproductive failure (Jadhav et al., 2007; Rapisarda et al., 2016). Hg exposure impairs the function of the nervous system and behaviour in mammals (Clarkson and Magos, 2006), determines a risk of reproductive and developmental disorders (Boujbiha et al., 2009; Scheuhammer et al., 2017). Vanadium compounds have been shown to inhibit or stimulate the activity of many DNA or RNA enzymes inducing several genotoxic and mutagenic effects (Stemmler and Burrows, 2001). Vanadium is released in the environment via combustion of fossil fuels such as coal and oil and through a range of industrial processes (Arena et al., 2015; Copat et al., 2012) characteristics of the industrial pole of Augusta-Priolo-Melilli.

Hepatic toxic thresholds for metals available in the literature were used to compare our data (Table 3). For the non-essential metals are suggested the following values: no greater than 30 ppm of Hg in mink, mustelids and carnivorous (Ma and Talmage, 2001); no greater than 13 ppm of Cd liver in cattle, sheep and pig (Reis et al., 2010); no greater than 10 ppm for Pb liver in small mammals (Hernout et al., 2016a); no greater than 8.7 ppm of As liver in wood mice and bank voles (Erry et al., 2000). The metal concentrations in bat tissues found in the polluted site are well below these toxicological values (10, 11, 39, 68-fold below for Hg, Cd, Pb, As, respectively) and the data from the control site are also well below the toxic thresholds (15, 37, 68, 77-fold below for Hg, Cd, Pb, As, respectively).

Toxic thresholds for metal concentrations in hair for small mammals are only available for Hg (Table 4). Nam et al. (2012) report a threshold of 10 ppm associated with neurobehavioral disorders, and Åkerblom and de Jong (2017) a threshold of 30 and

Table 2
Spearman correlation test of trace elements in fur versus liver. In italic are presented the significant relationships.

Area	As	Cd	Co	Cr	Cu	Hg
Palombara (Rho; p-value)	0.110; 0.467	*- 0.388; 0.008*	0.178; 0.238	0.077; 0.611	0.040; 0.792	0.054; 0.722
Pipistrelli (Rho; p-value)	*0.458; 0.001*	0.102; 0.485	0.157; 0.282	*- 0.309; 0.031*	0.066; 0.652	*0.627; 0.000001*
	Mn	Ni	Pb	Sb	Se	V
Palombara (Rho; p-value)	0.092; 0.544	0.095; 0.529	0.188; 0.210	0.255; 0.088	0.040; 0.791	*0.387; 0.008*
Pipistrelli (Rho; p-value)	0.090; 0.537	0.002; 0.989	0.301; 0.048	0.214; 0.139	*0.343; 0.016*	*0.492; 0.0003*

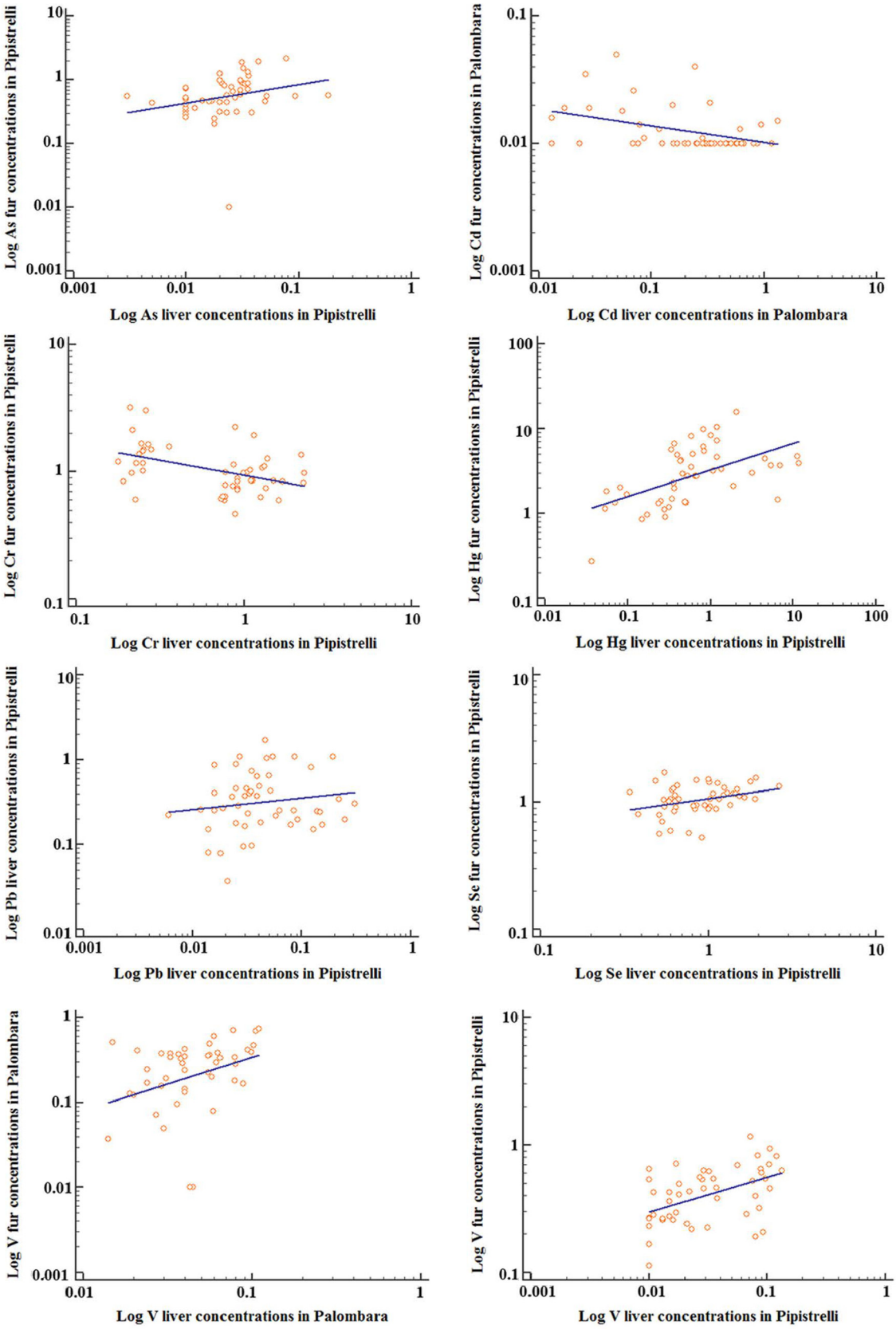

Fig. 4. Scatter diagrams and regression lines of metal concentrations with significant Spearman correlation between fur and liver. Concentrations were log transformed as $\log(y) = a + b \log(x)$.

Table 3
Metal concentrations (mg/Kg dry weight) detected worldwide in liver of different species of bats. *For metal concentrations in liver expressed as wet weight we converted the results in dry weight applying the conversion factor (CF = 4) given by Ma (1994). Toxic threshold or lower and upper range values are presented in table.

Liver	Area	As	Cd	Co	Cr	Cu	Hg	Mn	Ni	Pb	Sb	Se	V	References
Median	Palombara	0.128	1.136	0.364	3.52	29.99	2.98	35.84	0.128	0.252	0.040	2.708	0.16	Present study*
Median	Control Site, Pipistrelli	0.112	0.352	0.328	3.52	32.77	2.06	33.28	0.084	0.148	0.040	3.548	0.116	Present study*
Mean	Control Site, Virginia	–	–	–	–	–	5.86	–	–	–	–	–	–	Nam et al., 2012
Mean	South River, Virginia	–	–	–	–	–	71.9	–	–	–	–	–	–	Nam et al., 2012
Median	England and Wales	–	0.02	–	–	10.69	–	–	–	0.70	–	–	–	Hernout et al., 2016a
Range	Britain	–	0.83 −6.27	–	–	–	0.93 −3.0	–	–	1.16 −4.05	–	–	–	Walker et al., 2007
Mean	Iberian Peninsula	–	–	–	–	–	0.72	–	–	–	–	–	–	Lisón et al., 2017*
Range	Czech Republc	–	–	–	–	–	–	–	–	0.56 −10.0	–	–	–	Pikula et al., 2010*
Range	Control Site, Brazil	–	<loq − 4.8	–	<loq- < loq	15.5 −20.3	–	26.3 −34.8	<loq − 4.2	<loq- < loq	–	–	–	Zocche et al., 2010
Range	Sideròpolis, Brazil	–	<loq- 4.0	–	<loq- 5.7	23.2 −28.8	–	13.6 −59.5	<loq- 8.6	<loq-5.8	–	–	–	Zocche et al., 2010
Range	New York	–	0.21 −8.61	–	0.33 −5.25	17.0 −43.0	0.37 −8.34	23.2 −26.06	–	0.18 −21.4	–	2.6−8.96	–	Courtin et al., 2010
Range	Starlight Cave, Australia	–	0.82 −0.95	0.062 −0.063	0.31 −0.35	24.2 −25.1	0.28 −0.35	23.2	–	0.103 −0.109	–	2.6−2.7	0.011 −0.016	Allison et al., 2006
Range	Bat Cave, Australia	–	0.09 −0.10	0.094 −0.103	0.31 −0.35	32.7 −34.9	0.39 −0.47	25.7 −29.1	–	0.246 −0.279	–	4.1−4.7	0.013 −0.022	Allison et al., 2006
Mean	Germany	–	0.21	–	2.0	–	–	–	–	4.4	–	–	–	Streit and Nagel, 1993a
Mean	Germany	–	1.5	–	4.6	–	–	–	–	12.2	–	–	–	Streit and Nagel, 1993b
Mean	Sweden	–	3.3	–	–	–	3.6	–	–	–	–	–	–	Gerrell, and Lundberg, 1993
Range	Egypt	–	0.10 −0.22	0.10 −0.11	1.48 −3.78	4.91 −11.44	–	4.89 −8.82	0.44 −1.26	0.58 −1.12	–	–	–	Mansour et al., 2016
Mean	Mexico	–	6.5−8.0	–	–	13.1 −27.4	–	1.40 −3.68	–	0.57 −1.25	–	–	–	Méndez and Alvarez-Castañeda, 2000
Toxic threshold/Lower and upper range values		8.7[a]	>13[b] −105[c]	–	4[d]	20−30[e]	30[f]	–	–	10[c]	–	1.5−15[b]; 2.2[g]	–	–

[a] As upper range value in wood mice, bank voles in a contaminated site, from Erry et al. (2000).
[b] Toxic threshold for Cd suggested for Cattle Sheep and Pig, from Reis et al. (2010.
[c] Structural and functional kidney damage, from Hernout et al., 2016a.
[d] Toxic threshold for Cr as suggestive of environmental contamination, from Sheffield et al. (2001.
[e] Lower and upper level concentrations for essential trace metals, from Hernout et al., 2016a.
[f] Toxic threshold fo Hg suggested for intoxication in mink, mustelids, carnivorous, Ma and Talmage, 2001.
[g] Upper range value for Se, from Sheffield et al. (2001).

100 ppm associated with damage of mitochondrial DNA and impairment of neurological function, respectively. The data reported in this study are well below the lower toxic thresholds proposed (6 and 4-folds below in Palombara and Pipistrelli,

Table 4
Metal concentrations (mg/Kg dry weight) detected worldwide in fur of different species of bats. Toxic threshold and upper range values are presented in table. * Results are derived from the box plots.

Fur	Area	As	Cd	Co	Cr	Cu	Hg	Mn	Ni	Pb	Sb	Se	V	References
Median	Palombara, Italy	0.434	0.015	0.023	0.868	5.206	1.821	2.193	0.249	0.240	0.044	0.748	0,298	Present study
Median	Control Site, Pipistrelli, Italy	0.554	0.014	0.061	0.987	7.349	2.458	2.917	0.433	0.287	0.054	1.061	0,427	Present study
Median	England and Wales	–	0.100	–	–	14.96	–	–	–	28.8	–	–	–	Hernout et al., 2016a,b
Mean	Iberian Peninsula	–	–	–	–	–	1.130	–	–	–	–	–	–	Lisón et al., 2017
Range	Atlantic Canada	–	–	–	–	–	3.50−28.2	–	–	–	–	–	–	Little et al., 2015
Range	Northeast U.S.	–	–	–	–	–	0.02−0.74	–	–	–	–	–	–	Yates et al., 2014
Range	Giessen, Germany	–	0.14 −0.81	–	–	36.2 −123	–	4.96 −83.7	–	1.14 −34.2	–	–	–	Flache et al., 2015
Mean	Southern Sweden	–	–	–	–	–	1.5	–	–	–	–	–	–	Akerblom et al., 2017
Median	Central Hesse, Germany	–	0,16 −0,2*	–	–	30-70*	–	1,0−50*	–	0,1-0,3*	–	–	–	Flache et al., 2018
Toxic threshold/Upper range value		–	–	–	–	–	–	10[a] - 30[b]- 100[c]	–	–	–	–	>4[d]	–	–

[a] Toxic threshold for neurobehavioral disorders, from Nam et al. (2012).
[b] Toxic threshold for damage of mitochondrial DNA, from Åkerblom and de Jong, 2017
[c] Toxic threshold for impairment of neurological function, from Åkerblom and de Jong, 2017.
[d] Upper range value or heavy metal poisoning in Cattle Sheep and Pig, from Reis et al. (2010).

respectively).

In contrast to toxic elements, essential elements are functional, structural, and regulatory components of numerous biomolecules in metabolism in living organisms and they must be present at a specific concentration to ensure a good function of the metabolism (Windisch, 2002). Essential metal concentrations in fur and Se concentrations in liver were found significantly higher in bats sampled in the control area than in Palombara. These findings point towards a better nutritional status of the bats living in Pipistrelli cave, although further clinic-pathological studies involving immunoassays or metabolomics should be investigated to provide a better understanding of adverse effects of metal contamination in bats.

Metals such as Co, Cr, Cu, Ni, Se and V, have a specific role in an enzyme or cofactor pathway depending on the chemical species. A deficiency or an excess of these metals could produce a disease or impairment of function. V and Cr may play crucial roles in controlling blood glucose concentrations (Panchal et al., 2017). V concentration is largely influenced by the geographical location. For example, groundwater samples from volcanic areas are characterized by high concentrations of V, ranging from 0.05 to 2.47 mg/L (Arena et al., 2015). Nevertheless, high doses of V exposure are toxic, and inhalation exposure to V adversely affects the respiratory system (Ngwa et al., 2017). Many studies showed that Cr(III) improved insulin sensitivity and blood glucose levels in animals and humans with impaired glucose tolerance, insulin resistance and diabetes (Sharma et al., 2011; Tang et al., 2015). The use of energy nutrients can be increased by Cr (III) by influencing the activity of insulin receptors and thus, accelerate the loss of body weight and affects the body composition in rats (Kuryl et al., 2006). Co has a biological necessary role as metal constituent of vitamin B12, but an excessive exposure of Co has been shown to induce various adverse health effects (Leyssens et al., 2017). Nickel is an essential micronutrient which works as a cofactor for urease, hydrogenase, superoxide dismutase, and glyoxalases and plays a role in nitrogen metabolism (Küpper and Kroneck, 2007). Among the metals found with concentrations higher in fur of Pipistrelli' bats than Palombara' ones, Cu and Se are essential microelements found in all living organisms. Cu is required for survival and serves as an important catalytic cofactor in redox chemistry for proteins that carry out fundamental biological functions, important in growth and development. Cu, both with Mn and Zn, are essential to activate the SOD antioxidant defence, and the dietary intake of antioxidant nutrients may reduce oxidative stress, once they are able to eliminate free radicals in a direct way, as is the case of vitamins, or in an indirect way, through minerals, which act as co-factors of antioxidant enzymes (Ferrante et al., 2017a). Some metals could occur naturally in the area of Pipistrelli, because soils derived from mafic igneous rocks are known to be richer in bearded-trace metal mafic minerals and contain a higher concentration of metals such as Cr, Ni, Co and V (dos Santos et al., 2017). Although this area does not receive any direct anthropic contamination, the atmospheric transport and deposition of trace elements and nutrients can occur (Jickells et al., 2016). The metals most implicated in this global-scale atmospheric pollution are V and Ni, which are the most abundant metals present in crude oil, commonly in concentrations that exceed 1000 ppmV and 100 ppmNi (Moreno et al., 2010).

The following upper range values are suggested for the essential metals (Tables 3 and 4): 4 ppm of Cr in liver and 2.2 ppm of Se in liver in Rodentia and Lagomoroha as suggestive of environmental contamination (Sheffield et al., 2001); 30 ppm of Cu in liver in small mammals (Hernout et al., 2016a; Ma and Talmage, 2001). The Cr concentrations measured in this study are close to the upper range value in both populations (1.13-fold below). Se concentrations in bat liver from this study are slightly higher than the upper range value suggested by Sheffield et al. (2001) (1.08 and 1.4-fold above in Palombara and Pipistrelli, respectively), but below the upper range value of 15 ppm suggested by Reis et al. (2010) for cattle, sheep and pig. Concentrations of Cu in bat liver from this study are within the upper range value (30 mg/kg d.w.) suggested for shrews and moles (Ma and Talmage, 2001) in both populations analysed. Another study reports some poisoning concentrations of mineral elements and heavy metals in fur, blood and other organs of cattle, sheep and pig (Reis et al., 2010) and suggested a toxic value of Se above 4 ppm for fur. Here again, Se fur concentrations found in bats from Palombara and Pipistrelli are 5 and 4-fold below these toxic thresholds, respectively. Overall the concentrations of metals determined in bat samples from Sicily are lower than the toxic thresholds or the upper range values available (for essential elements) in the literature for small mammals and other vertebrate species.

4.2. Trace element correlations between fur versus liver

Significant positive correlations between tissues were found only for the toxic metals As, Hg and Pb in bats sampled from Pipistrelli (Table 2). Interestingly, no significant positive correlation between tissues was observed for these metals in bats from Palombara cave (Table 2). This could be explained by the greater chronic exposure and accumulation of these metals over time. Among the metals analysed, only V follows a positive correlation between tissues in both populations (Table 2). Similar exposure resulted suggest a continuous and chronic exposure of V, which could be a similar ratio of atmospheric exposure of V in both areas. The positive correlation found between concentrations of V in fur and liver is slightly stronger in Pipistrelli than in Palombara (Table 2). Another positive correlation was observed between liver and fur concentrations of Se in bats from Pipistrelli (Table 2).

A different trend was observed for the toxic metal Cd, with a negative correlation between fur and liver concentrations in bats from Palombara (Table 2). Cd body burdens in liver and kidneys contain about 50% of the body's accumulation of cadmium (ATSDR, 2008; HSDB, 2006), with a long half-lives (4 and 19 years in liver, 6–38 years in kidney) reflecting the fact that mammals do not have effective pathways for cadmium elimination (ATSDR, 2008). With prolonging the exposure to Cd, its level in the kidneys exceeds that in the liver (Zabulyte et al., 2007) and it is eliminated from the body very slowly and in almost equal parts with feces, urine, hair, and sweat (Su et al., 2017). Thus, in bats from Palombara, the higher Cd concentrations detected in liver may lead to a progressive Cd excretion, and here it is highlighted by a negative regression with hair Cd concentrations. Around 50% of the Cd concentrations determined in fur samples were below the LOD. These results could explain our weak negative correlation.

Another negative and weak correlation between fur and liver concentrations of Cr was also observed in bats form the control site (Table 2). The concentrations of Cr in fur were significantly higher in bat sampled from the control site than the polluted site ($p < 0.001$) (see section results). We suggest that a greater bioavailability of the essential Cr in Pipistrelli, allows its faster process in the liver for different biological functions and will not accumulate, whereas in fur the concentration can increase with a constant flow for its removal. Here, we supposed that an adequate and sufficient uptake of the metal allows a homogeneous metal distribution between tissues, although the knowledge about detoxification and excretion mechanisms and patterns of metals in bats is still very limited.

A number of recent studies used hair analysis to biomonitor metal exposure in bats (Lison et al., 2017; Flache et al., 2018). However, only a few studies have demonstrated their utility by comparing metal body burdens in bat fur versus in internal organs,

particularly key organs involved in bioaccumulation and detoxification process (i.e. kidneys, liver) (Hernout et al., 2016a,b; Lison et al., 2017; Nam et al., 2012). In accordance with the results from this study, others studies showed overall significant and positive correlations between fur and liver concentrations of non-essential metals. For essential metals, the regulation by homeostatic mechanisms suggests an effective process (D′ Havé et al., 2006). The strong positive associations between fur and liver concentrations in this study (Table 2) are comparable with previous reported results: for Pb in the wood mice (*Apodemus sylvaticus*) (Tête et al., 2014), and for Pb and As in the European hedgehog (*Erinaceus europaeus*) (D'Havé et al., 2006). For Hg, Nam et al. (2012) showed positive relationships between total Hg concentrations in fur versus brain tissues.

Interestingly, the significant positive relationships in this study (for Hg, As, Pb, V and Se - except for V in Palombara), were only showed for the control site (Table 2). This suggests that the different patterns of accumulation in diverse tissues follow different mechanisms when subject to a high exposure. However, in contrast to our results, associations between Hg concentrations in fur and liver of *Miniopterus schreibersii* sampled from a special Area of Conservation being part of the Natura 2000 network, - and therefore an area not believed to be polluted - did not show any significant relationships (Nam et al., 2012). This study was using a limited number of samples ($n = 24$), which could explain this results (Nam et al., 2012). A larger number of bat samples ($n = 191$) from across England and Wales did not show a significant relationship between concentrations of Pb in liver and fur, but did for Pb and kidneys (Hernout et al., 2016a,b). These samples were collected across a gradient of metal concentrations. The high variability in these results might be explained by a range of variables such as age, diet and moulting stage, as well as the levels of contamination in their environment (soils, invertebrates), which can impact the levels of metals contained in fur or hair. However, it would be interesting to identify whether the degree of contamination and environmental exposure of metals has an effect on the relationships between fur versus internal concentrations. Studies using non lethal proxy such as fur and hair as a biomarker in bats and wildlife species are increasing and are to be strongly encouraged, and it would be valuable to provide the optimal conditions to biomonitor bats for environmental pollution.

4.3. Trace element comparison with literature data

In literature, liver and other internal organs concentrations of metals are a more in-depth discussed than fur (Allinson et al., 2006; Courtin et al., 2010; Gerrel and Lundberg, 1993; Hernout et al., 2016a; Lisón et al., 2017; Mansour et al., 2016; Méndez and Alvarez-Castañeda, 2000; Nam et al., 2012; Pikula et al., 2010; Streit and Nagel, 1993a, 1993b; Walker et al., 2007; Zocche et al., 2010). Nevertheless, As and Sb body burdens in bats were never studied in liver tissues, and only a few data are available for the other metals, especially for Co, Ni, Se and V. Therefore, this study is the first to present a multi element dataset for insectivorous in Europe, by comparing body burdens from a control versus a polluted site. Overall, results reported in literature are highly variable depending on the species and the geographical locations (Table 3). Regarding toxic metals, Cd concentrations in liver are highly variable, ranging from <lo to 8.61 mg/kg dw based on the species and the geographic area (maximum amount found in Northern long-eared - *Myotis septentrionalis*) with white-Nose Syndrome in the Northeastern United States (Courtin et al., 2010) (Table 3). We found Cd concentrations generally in the range or lower than literature findings (with 0.4 and 1.1 mg/kg dw, in Palombara and Pipistrelli, respectively) (Table 3). Hg concentrations in liver show a great variability depending on geographical location, and the results presented in this study present intermediate concentrations. Pb results reported in this study (0.3 and 0.1 mg/kg dw, in Palombara and Pipistrelli, respectively) are within the range of literature data (<loq - 21.4 mg/kg dw) (Table 3) and mostly lower than the average values reported from other studies (Table 3).

Regarding essential elements, only three studies report Co concentrations in liver, and values obtained from bats in Palombara and Pipistrelli are higher than concentrations observed in *Miniopterus schreibersii bassanii* and *Pipistrellus pipistrellus* from Australia and Egypt, respectively (Table 3). Concentrations of Cr in liver from this study are generally higher than other literature findings in several species of bats (Table 3) although, higher values were reported from Cr body burdens in liver from bats in from Brazil, USA and Egypt (Table 3). Regarding Cu, the range reported in literature is highly variable (ranging from 5 to 35 mg/kg) (Table 3), and our results were similar to the results reported from Zocche et al. (2010), Courtin et al., 2010 and Allinson et al., 2006 from Brazilian, North American and Australian bats (Table 3). Mn concentrations in liver from the Sicilian bats were generally higher than literature findings with the exception of fructivorous species collected near a coal mining area in Brazil (Zocche et al., 2010). However, our results were similar to the concentrations found in the bats from the control site (Zocche et al., 2010-Table 3).

Few studies reported metal concentrations in fur of bats and the results were limited to a group of metals, such as Cd, Cu, Mn, Pb and Hg (Åkerblom and de Jong, 2017; Flache et al., 2018, 2015; Hernout et al., 2016b, 2016a; Lisón et al., 2017; Little et al., 2015; Yates et al., 2014). Comparing our results with previously published data (Table 4), Hg bioaccumulation in fur follows the same trend of results reported from bats samples in unpolluted area. In one Hg polluted site in Canada, Little et al. (2015) found values significantly higher with a mean concentration value of 28.2 mg/kg compared to a control site. As well, Hernout et al. (2016a), by analysing fur of bats from England and Wales found values of Cd, Cu and Pb higher than the bat from Sicily. Another study conducted in two urbanized area of Germany reports Cd, Cu, Mn and Pb concentrations highly variable depending on the analysed species, and the values reported are higher than our results, with the exception of Pb revealed in species collected in Central Hesse, Germany (Flache et al., 2018), where concentrations are similar than that forum both in Palombara and Pipistrelli (Table 4).

Overall, our results were within the range or lower than previous reported results from the literature (Tables 3 and 4), and particularly lower when compared to studies reporting body burdens from bats or samples collected in polluted areas, especially for Cd, Hg and Pb (e.g. coal mining area in Brazil, bat cave in Australia with pesticide residues detected in bat cave and North America bats infected by the white nose syndrome) (Table 3). Given these results and our comparison with previous reported toxic thresholds data, our results do not show evidence that the bat population may have contaminated by metal pollution in Sicily. However, our results are showing a higher accumulation of metal in the polluted cave. In addition, the limitations of the toxicological thresholds need to be highlighted since they do not account for possible variation of vulnerability across various factors including the species, season, diet, and age, and the knowledge about the associated adverse effects and their mechanisms could be better understood. Further studies are to be encouraged to refine our knowledge on the potential metal toxicity of metal as well as other potential contaminants to bats using immunohistochemistry, metabolomics and neurochemicals biomarkers (such as in Nam et al., 2012), especially in the context of recent drastic declines of chiropteran species.

5. Conclusion

Metals concentrations in fur and liver of *M. myotis* sampled across two caves in southern Sicily highlighted a different pattern of accumulation of essential and toxic or non-essential metals. Results showed higher concentrations of essential elements in bats samples from the control site. Conversely a higher bioavailability of toxic elements was found in bats from Palombara cave, where bats have a hunting area extended within the boundaries of an industrial area. Positive correlations of metal concentrations between tissues were found primarily in bats from the Pipistrelli cave, confirming the utility of fur as a non-invasive proxy to biomonitor metal contamination in bats. The differences of metal concentrations between the sites could be explained by different environmental bioavailability of metals in the two populations' hunting area. Nevertheless, based on our results, we cannot consider Palombara population as polluted by metal contamination since their tissue concentrations are below toxic threshold or upper range values previously reported. However, we cannot exclude other kind of pollutants as potential stressors of the examined population.

Declaration of interest

None.

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